Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 24 No. 1 Ser. No. 82 2023

IRANIAN JOURNAL OF VETERINARY RESEARCH

Review Article

Camel semen collection, viscosity, and cryopreservation: a review

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10.22099/IJVR.2023.44924.6624

(Received 1 Oct 2022; revised version 19 Dec 2022; accepted 2 Jan 2023)

Abstract

Artificial insemination in camels remains undeveloped due to the difficulties in semen collection, semen viscosity, and semen cryopreservation. The semen collection procedure has been facilitated to some extent using camel phantom and/or possibly an intravaginal condom. Main reasons for semen viscosity in camelids have been unraveled and different mechanical and enzymatic approaches were used to alleviate this problem; however, there is still no conclusive protocol to safely remove semen viscosity completely. It seems that along with the problem of semen viscosity, semen cryopreservation in camels remains unresolved. As a result, there is no convincing report on successful and repeatable pregnancies following insemination with frozen semen in camel. This review gathered most of the information that appeared in the peer reviewed journals to highlight major problems in camel semen technology, including semen collection, semen viscosity, and semen cryopreservation.

Key words: Camel semen, semen collection, semen viscosity, sperm cryopreservation

Introduction

There is a great lag in the development of reproductive technologies in camels compared to other domestic animals. Semen technology, as the first generation of reproductive technologies, remained behind other generations of reproductive technologies like *in vitro* and *in vivo* production of embryos and cloning in camel. While AI with fresh and frozen semen is widely used in domestic animals, the progress of AI in camels is very slow. There is not a single camel bull stud in the world to collect and process semen for routine AI and cryopreservation successfully. Development of an artificial insemination network in camel depends on the ability:

a) To collect clean and viable semen safely and easily
b) To remove viscosity of semen without affecting sperm viability
c) To extend the non-viscous semen in a suitable extender for performing artificial insemination and/or semen cryopreservation

The present review has been prepared to elaborate on the current status of semen technology with respect to semen collection, viscosity, and cryopreservation in camel.

Semen collection

The first step in developing AI in camels is the ability to collect clean and viable semen. Semen collection in camel is a challenging procedure due to the mating in sternal recumbency, prolonged time of copulation, and possible injuries to the operator (Tibary and Anouassi, 1997). There are four main approaches for collecting semen in camels, including artificial vagina (Tibary and Anouassi, 1997; Mosaferi *et al*., 2005), electroejaculation (Tingari *et al*., 1986), phantom or dummy (Ziapour *et al*., 2014), and intravaginal condoms (Tibary and Anouassi, 2018; Mansour, 2022). These methods have advantages and disadvantages among which semen quality and animal welfare are the main challenges. Artificial vagina (AV) is the most common approach for semen collection in camels (Skidmore *et al*., 2020). Although AV simulates natural mating, the corresponding procedure in camel is tiring and hazardous to the operator mainly due to the prolonged time of semen collection and the position required collecting semen. More importantly, due to the several backward and forward movements of a bull during semen collection, the specimen is more likely to be contaminated (Ziapour *et al*., 2014). Electroejaculation is

another approach for collecting semen from camel bulls (Tingari *et al*., 1986). This method is not recommended for routine semen collection from valuable males. The procedure requires sedation or even anesthesia, and potentiates some risks to the life and welfare of the animal. Moreover, the quality of semen collected by an electroejaculator varies in volume and concentration (Tibary and Anouassi, 2018). Phantom could be a suitable replacement for live female camels for semen collection. Phantom eliminates the risk for the operator, facilitates semen collection procedure, and provides more natural conditions to collect good quality and clean specimens (Ziapour *et al*., 2014; Panahi *et al*., 2017). The main point in using phantom for semen collection in camels is the patience required for training the bull. Once a single bull is trained, it is possible to use this bull to stimulate others. Several tactics could be taken to train the bull to accept the camel dummy. To stimulate the bull camels, complete isolation of them from she-camels throughout the season, spraying urine of estrous she-camel around the back and perineal region of the camel dummy, and playing the recorded sound of she-camel during mating could be helpful. Recently, using intravaginal condoms was revisited for semen collection in camels (Tibary and Anouassi, 2018; Mansour, 2022). Major constraints in using condoms for semen collection in camels include problems associated with the installation of the device inside the vagina and its fixation around the vulva, and removing the installed condom due to clockwise and anti-clockwise movement of the penis. Moreover, due to causing the unpleasant condition for she-camel, using the condom device does not seem to receive animal ethics approval.

Semen viscosity

Camel semen is highly viscous by nature (Deen *et al*., 2003), with mass vibration at the initial raw semen assessment (Panahi *et al*., 2017) and prolonged liquefaction time of 18-41 h (Mal *et al*., 2016). Proteins belonging to the β-nerve growth factors family might be responsible for the liquefaction of camel semen (Mal *et al*., 2016). The viscous nature of the ejaculates makes the processing and pipetting of raw or even diluted semen very difficult. Besides, semen cryopreservation becomes unsuccessful because the viscous seminal plasma prevents penetration of cryoprotectant into the spermatozoa. The role of camel semen viscosity is not fully understood. It is postulated that semen viscosity might be required to prevent loss of sperm from the female reproductive tract (Kershaw-Young and Maxwell, 2012). Regardless, semen processing, evaluation, cryopreservation, and even AI require complete liquefaction. The causes of semen viscosity and its elimination have been a challenging subject in the world of camelid research. During the early sixties, it was suggested that mucopolysaccharide secreted by bulbo-urethral gland might be responsible for camel semen viscosity (Perk, 1962). However, recent studies suggested that these chemicals, renamed glycosaminoglycans, may not be the main source of semen viscosity in camelids (Kershaw-Young and Maxwell, 2012; Kershaw-Young *et al*., 2013). There is sufficient evidence to accept that proteins within seminal plasma such as mucin may be responsible for viscosity in camelid semen (Kershaw-Young and Maxwell, 2012). The viscosity of semen can be reduced by adding cysteine protease, such as papain, present in papaya (Kershaw-Young *et al*., 2013, 2017; Monaco *et al*., 2016; El-Bahrawy *et al*., 2017) or ficin, present in fig (Keshavarz *et al*., 2016) to semen. Following ficin treatment and centrifugation, round pellet forms at the bottom of the conical tube in association with the ability to separate supernatant from the pellet. However, in an untreated-centrifugated specimen, an oblique and sticky pellet forms at the wall of the tube and is removed with the supernatant during withdrawal (Keshavarz *et al*., 2016). The positive and negative effects of enzyme treatment on semen occur fairly quickly. In fact, the amount of time required to remove semen viscosity by the enzyme is fairly similar to the time that the adverse effect on sperm viability occurs by the enzyme. Attempts have been conducted to neutralize the enzyme following partial semen liquefaction (Kershaw-Young *et al*., 2017; Malo *et al*., 2017b); however, there is no convincing so far report on the benefit of such treatment for semen cryopreservation and successful pregnancy following AI.

Apart from the enzymatic approach to improving the rheological characteristics of camel semen, mechanical and ultrasonic approaches were also investigated. Stirring of camel semen at a very low speed (150 rpm) for 15 min (Mosaferi *et al*., 2005), gentle pipetting of diluted semen (Morton *et al*., 2008), and passage of semen back and forth through a needle could also be used to reduce semen viscosity (Santiani *et al*., 2005). Ultrasound wave (40 kHz) was also imposed to Dromedary camel semen for 2 min, interspersed for 2 min, and repeated 4 times to reduce semen viscosity (Rateb, 2016; El-Bahrawy *et al*., 2017). However, the safety and feasibility of such a method were not confirmed in other studies. While all of these methods could be simple and relatively effective, they do not completely eliminate semen viscosity. It is suggested that camel sperm could be extracted from seminal plasma without enzymatic extraction using a combination of pipetting and colloid single layer centrifugation without detrimental effect on sperm quality (Malo *et al*., 2017a, 2018a; Morrell *et al*., 2021). This method could be helpful to achieve a low sample of good quality sperm for *in vitro* embryo production; however, it did not provide a promising result in terms of semen cryopreservation.

Semen cryopreservation

The progress in camel semen cryopreservation has been slow compared to other livestock species. This, in turn, prevents extensive use of artificial insemination in camels. To date, there is only one report on pregnancy following AI with frozen-thawed semen in dromedary

camel (Deen *et al*., 2003) and some reports in Bactrian camel (Chen *et al*., 1984, 1985; Zhao *et al*., 1994, 1996). There are no other reports on successful pregnancy in camels. Deen *et al*. (2003) reported a pregnancy rate of 0/10, 1/13, and 4/10 following insemination with liquid semen, frozen-thawed semen, and whole semen in dromedary camel, respectively. The later investigators considered 20% post-thaw motility as successful cryopreservation; whereas, it is commonly accepted that 40% post-thawed progressively motile sperm could be considered as a minimum standard for frozen semen to be used in extensive AI programs.

If a clean, viable, and non-viscous semen sample is an initial step, a suitable extender is the next step toward the development of extensive AI programs. There are few suitable and chemically defined extenders for semen preservation in camel. Lactose and sucrose have been used to preserve Bactrian camel semen (Chen *et al*., 1984, 1985; Zhao *et al*., 1994, 1996; Niasari-Naslaji *et al*., 2006a, b, 2007a). These two extenders were not efficient even for chilled storage of Bactrian camel semen (Niasari-Naslaji *et al*., 2006a, b, 2007a); however, they were reported to be good for cryopreservation of Bactrian camel semen, resulting in pregnancy rates of 86-100% following insemination with frozen semen (Chen *et al*., 1984, 1985; Zhao *et al*., 1994, 1996). Laiciphos, Androhep, and glucose-EDTA have also been investigated for the preservation of camel semen (Sieme *et al*., 1990), with no exact quantification of the sperm viability parameters. Tris extender was suggested to be better than lactose (Vyas *et al*., 1998) and Bicephos (Deen *et al*., 2004) for the chilled storage of camel semen. Green buffer (Niasari-Naslaji *et al*., 2006a; Skidmore *et al*., 2013) and INRA 96 (Morton *et al*., 2013; Malo *et al*., 2020) were successfully used to preserve camel semen. Chemically defined tris-based extender named "SHOTOR" diluent was used successfully to store Bactrian camel semen in chilled and frozen states (Niasari-Naslaji *et al*., 2006a, 2007b, 2008). However, the post-thaw motility of Bactrian camel sperm using SHOTOR diluent did not reach 40% to produce a successful pregnancy. We found that, SHOTOR diluent was not a proper extender to preserve Dromedary camel semen. Therefore, we introduced new extender named "HASHI" diluent for chilled storage of Dromedary camel semen (Panahi *et al*., 2017). HASHI diluent consists of 60% SHOTOR diluent, 20% pigeon plasma egg yolk, and 20% camel skim milk (Panahi *et al*., 2017).

Several experiments were conducted to determine the type (Malo *et al*., 2017b) and concentration (Niasari-Naslaji *et al*., 2007b; Malo *et al*., 2017b) of cryoprotectants, dilution rate (Malo *et al*., 2017a), equilibration time (Malo *et al*., 2017b), the speed of cooling from room to 4°C (Niasari-Naslaji *et al*., 2007b), freezing rate (Malo *et al*., 2018b), thawing rates (Malo *et al*., 2018b), addition of antioxidants (Medan *et al*., 2008; Malo *et al*., 2019, 2020), and surfactants (Niasari-Naslaji *et al*., 2008) to cryopreserve camel semen. Unfortunately, the main obstacle to get meaningful and conclusive results in the majority of studies was the inability to safely remove the viscosity of camel semen completely. Moreover, in several studies, the investigators reported the total motility and or very low progressive forward motility of sperm, which is not a good indicator of successful cryopreservation or successful AI using frozen semen.

Conclusion

Although there is considerable progress toward collecting good quality semen in camel using phantom or intravaginal condom and providing a sound extender to preserve liquid stored camel semen, to date, to the best of my knowledge, there is no solid, convincing, and repeatable result on the production of healthy and live birth of camel calf following insemination with frozen-thawed semen. It seems that still the main constraint preventing successful semen cryopreservation in camels is semen viscosity. Therefore, several investigations with conclusive outcomes are required to solve the problem of semen viscosity prior to further attempts for cryopreservation of camel semen.

Acknowledgement

The author wishes to dedicate this review article to his mentor, Dr Lulu Skidmore, the scientific director of Camel Reproduction Center, Dubai, UAE, whom I learned the first steps in understanding the reproduction of camel in 1999. Since then, we became close friend. I wish her a very happy, prosperous life.

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